THE EFFECTS OF DIFFERENT ESTRADIOL DELIVERY METHODS ON PLASMA ESTRADIOL LEVELS IN MICE

An Undergraduate Research Scholar

by

KATHERINE MARIE OETKEN

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ABSTRACT

Estrogen Levels in Mice with Synthetic Estrogen Pellet Implant.
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Katherine Marie Oetken
Department of Nutrition and Food Science
Texas A&M University

Research Advisor: Dr. Clinton Allred
Department of Nutrition and Food Science

Evidence suggests that estrogen plays a role in decreasing the risk of developing colon cancer. Numerous animal studies demonstrate possible mechanisms to explain how estradiol (E_2) prevents this disease. Many of these studies involve removing the ovaries of mice and replacing E_2 at a controlled dose for the length of the study. One method of E_2 delivery involves compacting E_2 and cholesterol into a pellet and implanting it subcutaneously on the back of the animal. While the release patters of silastic implants have been studied previously, the compressed powder pellets release patterns has not. It is unclear if the results of studies using this hormone delivery method come from a steady release of the E_2 over the entirety of the study or if it is the result of a giant influx of E_2 levels just at the beginning. This ten-week experiment compared the amounts of E_2 released from an E_2 compressed powder pellet, a silastic E_2 implant and a control cholesterol implant. To observe the amount of estradiol released, female mice were first ovariectomized and received either a treatment of: E_2, control, or a silastic implant of E_2. Each week following the surgery, a group of mice were sacrificed from each of the three treatment groups. The blood was tested using an ELISA test to determine the levels of estradiol. The results showed that there was peak of E_2 directly following the implantation of the pellet and a week after implanting the silastic E_2, but the levels then remained constant.

ACKNOWLEDGMENT

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NOMENCLATURE

OVX	Surgery to remove uterus
E2	Estrogen

CHAPTER I

INTRODUCTION

Challenges in current research

Research has found a correlation between estradiol (E_2) and a reduced risk of colon cancer. The Women's Health Initiative study compared the risk of postmenopausal women who received hormonal replacement treatment with a placebo group and found that those who did receive the hormone therapy were more resistant to colon cancer [1]. Similar results have been observed in animal studies. One study treated a group of mice with E_2 then exposed them to carcinogens, and found the E_2 group to have fewer tumors than animals not receiving E_2 [2]. The mechanism as to how the E_2 protects against colon cancer is not fully understood. There is data to suggest that the E_2 plays an important role in inducing apoptosis, programmed cell death, of non-malignant colonocytes before they can develop into cancer. E_2 is also suggested to aid in repairing DNA double strand breakage. Since the mechanism of how E_2 protects against cancer is still developing, it is important to have accurate research on the topic. A common way to study this topic is through the use of animal models.

Controlling concerns

To better understand the protective role of E_2 observed in animal models, it is necessary to know the rate of exposure to E_2 over time in mice supplemented with E_2. The first step in many animal studies investigating the effects of the hormone estrogen is removing the ovaries via an ovariectomy. This ensures that the animals will not be producing significant levels of E_2 *de novo*. Once the ovaries are removed, E_2 implants are used to control the amount of E_2 the animal is

exposed to. Commonly, the E_2 implant is administered subcutaneously either in the form of an E_2 compressed powder pellet or a silastic E_2 implant. The silastic E_2 is more widely used and is a clear silicone tube that has a semi-permeable membrane that allows for the E_2 to diffuse through the tube and into the subject. The silastic implant has previously been observed to deliver hormones at a slow, constant, rate. The E_2 pellets are compressed E_2 mixed with cholesterol that is pressed into a cylinder form. There is no data showing the rate at which the E_2 is released from the pellet however it is speculated that the rate is a constant. Both forms of the E_2 implants will release E_2 for several weeks and are generally replaced every 8 weeks of a study to ensure constant E_2 exposure. To test the hypothesis of how the E_2 is released from the compressed powder pellet, the present study took weekly samples from mice that had been ovariectomized and received either an E_2 pellet, a silastic E_2 implant or a cholesterol control pellet to compare the plasma levels of E_2 and observe the rate of E_2 release.

CHAPTER II

METHODS

Collecting the data

Animals: Female c57bl/6 mice were ovariectomized on day 0 and implanted with one of three treatments at the base of the neck; a control compressed cholesterol pellet, a 0.5 mg E_2 + 19.5 mg cholesterol compressed powder pellet or a 0.5 mg E_2 + 19.5 mg cholesterol silastic implant. It's important to notice that the concentration of estradiol is the same for both the E_2 pellet and silastic E_2 groups. The mice were then separated into cages based on which week they would be sacrificed, with each cage having mice from all three treatments. Mice were sacrificed weekly by ketamine injection and blood was collected via cardiac puncture. Immediately after sacrifice, the uterus and colon were removed and the uterine weight and colon length were measured. Plasma was extracted from whole blood by centrifuging the samples at 2500 RPM for 15 minutes and then aliquoted prior to being stored at -20°C. Plasma levels of E_2 were measured from these samples using an enzyme linked immunosorbance assay (ELISA.)

Enzyme-linked immunosorbent assay (ELISA): The amount of estrogen in the plasma was determined using an Estradiol EIA Kit (Cayman Chemicals.) This is a competitive assay that determines estradiol levels based on absorbance. It is based on competition between estradiol and an estradiol-acetylcholinesterase conjugate for a limited amount of Estradiol antiserum. To begin, plates are previously coated with mouse monoclonal anti-rabbit IgG and blocked with proteins. The antiserum-estradiol complex will bind to the monoclonal anti-rabbit IgG.

The plates will then be washed to discard any unbound reagents and Ellman's Reagent will be added. This enzymatic reaction well reveal a yellow coloring that is directly proportional to the amount of Estradiol Tracer bound to the well. This amount of Estradiol Tracer is then inversely proportional to the amount of free estradiol present.

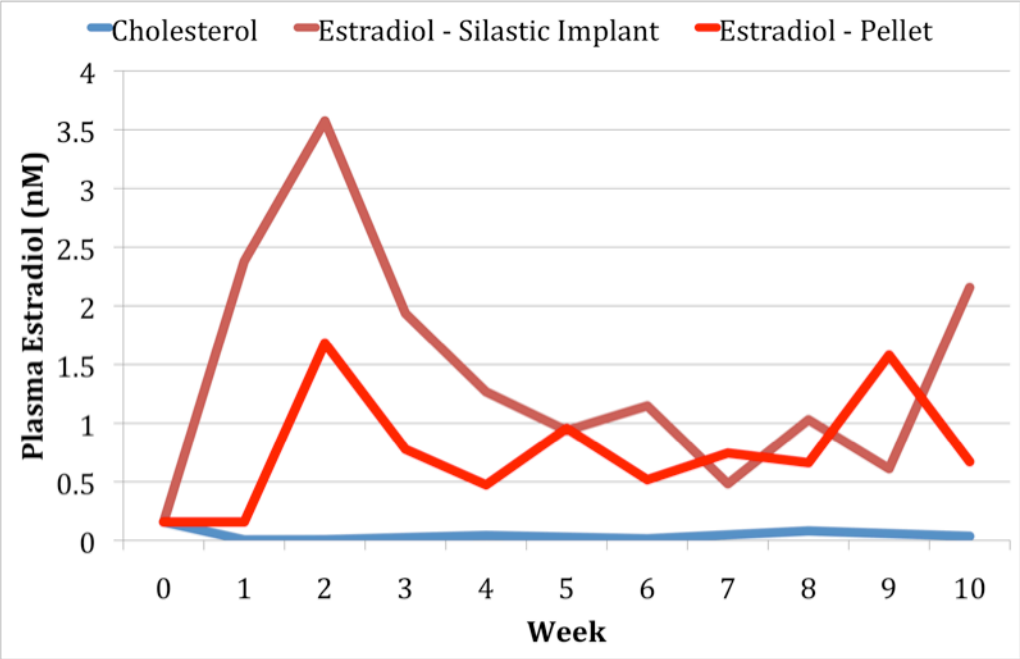
CHAPTER III

RESULTS

Data collected

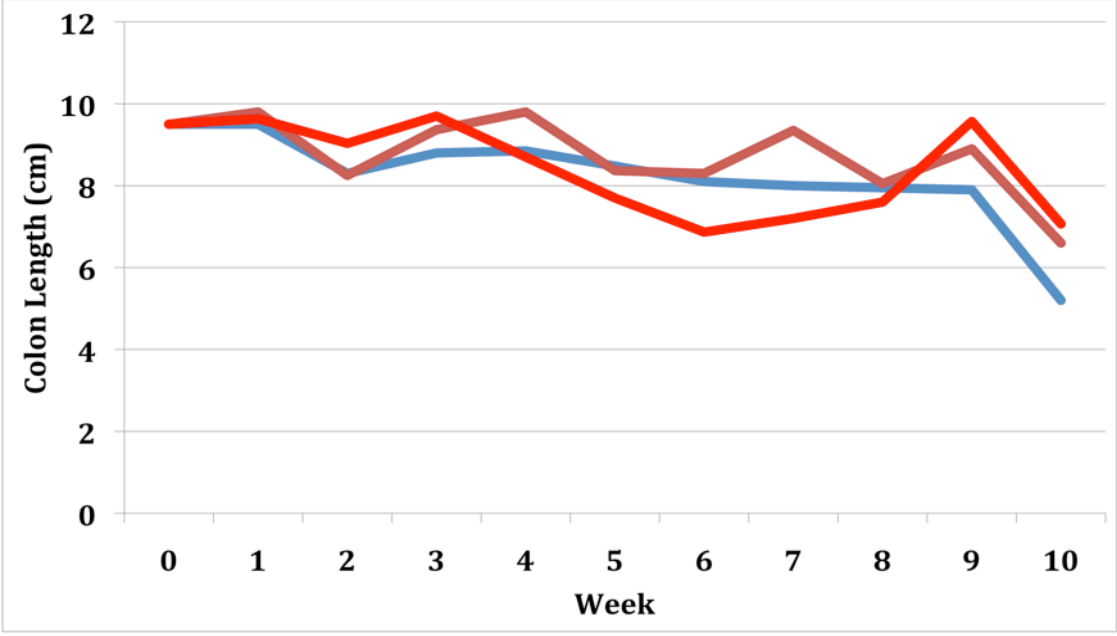
A total of 60 mice were collected for use in this study all of them were female c57bl/6 mice. One of those died premature to being sacrificed and was not included in the analysis. 59 mice remained to be analyzed. There were weeks with no control mice sacrificed and to get the data for that week it was averaged with the surrounding weeks. The data collected showed a peak in estradiol levels directly following the estradiol pellet implants, and a peak in estradiol levels a week after the silastic estradiol was implanted. Both of these treatments quickly went down to a steady release for the remainder of the study. The colon length showed no difference between any of the three treatments, however in all three treatments during the last week it's length did decrease. The uterus weight increased significantly with the two estradiol groups and was much smaller in the control group.

Figure 1



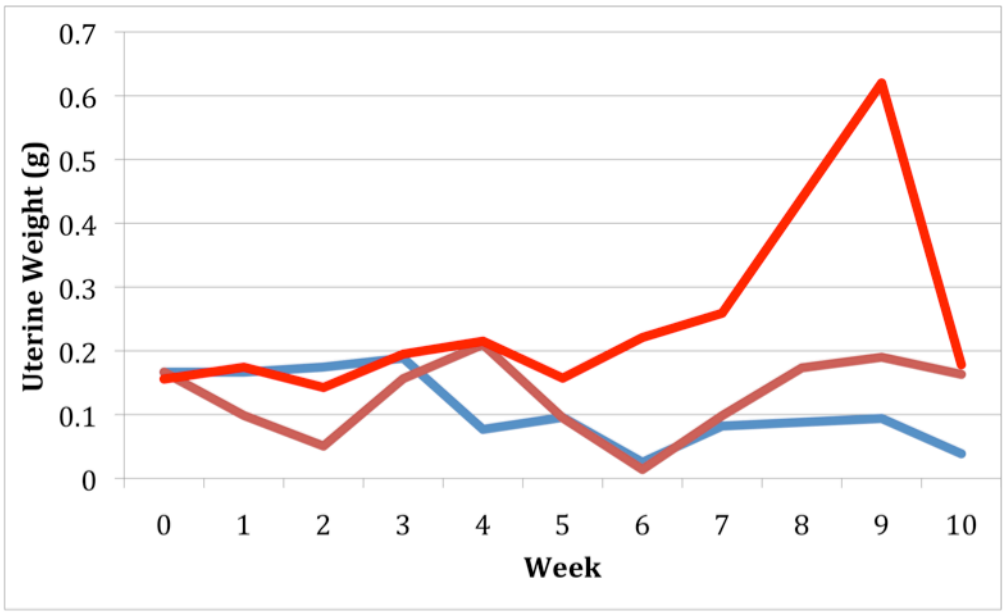
E_2 Plasma levels caused by different treatment. Values are the mean of estradiol in plasma .

Figure 2



Colon lengths caused by different treatment. Values are the mean of the colon length measured in cm.

Figure 3



Uterine weight caused by E_2 treatment. Values are the mean of the uterus weight.

CHAPTER IV

CONCLUSION

The final results supported the original claim that the E_2 pellet releases estradiol in a constant amount. For women an estradiol level between 1-2nM is physiologically common during a normal menstrual cycle, and was what the study hoped to achieve in the mice model. The data supported this claim and showed that most the E_2 pellet and silastic E_2 maintained estradiol levels between .7-1.2nM for the duration of the study. This confirms that the results of studies that use the E_2 pellets are not from high estradiol levels, and instead from the estradiol itself. In this experiment, both the E_2 pellet and silastic E_2 groups had a spike in plasma estradiol levels following implantation. The E_2 pellet's spike was immediately following the implant, and the silastic E_2 spike occurred a week following. After this spike, both released a steady amount of estradiol. This steady release of estradiol was the desired outcome. In addition to studying the plasma E_2 levels the colon lengths and uterus weight was measured to observe the effects. The colon length remained constant for the majority of the study until the final week when there was a slight decrease in length. This suggests that estradiol has little effect on the colon length since in both the E_2 group and cholesterol group there was no noticeable difference. Finally, as expected the E_2 treated groups had larger uterine weights than the control group due to atrophy of the uterus in the absence of E_2.

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